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Indian Standard

SPECIFICATION FOR BAKERY SHORTENING

(First Revision)

Second Reprint OCTOBER 2008
(Including Amendment No. 1)

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BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

Gr 7 + Gr 1 *December* 1986

Indian Standard

SPECIFICATION FOR BAKERY SHORTENING

(First Revision)

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(*Continued on page* 26)

AMENDMENT NO. 1 JULY 1994
TO
IS 10634 : 1986 SPECIFICATION FOR BAKERY SHORTENING

(First Revision)

(*Page* 4, *clause* 3.1, *line* 1) — Insert 'of white to pale yellow in colour' *before* 'clear in appearance'.

(*Page* 5, *clause* 3.1, *line* 5) — Insert 'rancidity or staleness' *before* 'foreign odour or taste'.

(*Page* 5, *clause* 3.4) — Substitute the following for the existing clause:

'The material shall contain such quantity of refined sesame oil (*see* IS 547 : 1968), which is sufficient to ensure that when the material is mixed with refined groundnut oil in the proportion of 20 : 80, the red colour produced by the Baudouin test shall not be lighter than 2 red units in 1-cm cell on Lovibond scale when tested as prescribed in Appendix A.'

(*Page* 5, *clause* 3.5) — Substitute the following for the existing clause:

'The material shall be free from non-edible oils, mineral oil, castor oil and animal fats when tested by the methods prescribed in Sections 9, 10, 12, 14, 15 and 18 of IS 548 (Part 2) : 1976.'

NOTE — Section 18 of IS 548 (Part 2) : 1976 may be used for detecting the presence of animal fat in bakery shortening. Section 21 of IS 548 (Part 2) : 1988 shall be used for confirmatory testing.'

[*Page* 6, *Table* 1, *Sl No.* (iii)] — Substitute the following for the existing matter:

'iii) Unsaponifiable matter, percent by mass, *Max* (*see* Note)	2.0	—	8

NOTE — In case of bakery shortening where proportion of rice bran oil is more than 30 percent by mass, the unsaponifiable matter, percent by mass, *Max* shall be 2.5 percent provided the quantity of rice bran oil of such bakery shortening is declared on the label.'

[*Page* 6, *Table* 1, *Sl No.* (vii)] — Add the following new item:

(1)	(2)	(3)	(4)
viii)	Nickel, ppm, *Max*	1.5	E

Price Group 1

[*Page* 7, *clause* 5.1(h)] — Renumber the existing as 5.1 (j) and include the following as 5.1 (h):

"Every package of bakery shortening made from more than 30 percent rice bran oil shall bear the following label:

This package of bakery shortening is made from more than 30 percent rice bran oil by mass."

(*Page* 25, *Appendix* F) — Add the following new Appendix:

'APPENDIX F

[*Table* 1, *Sl No.* (viii) *and clause* 7.1]

DETERMINATION OF NICKEL IN BAKERY SHORTENING

F-0 Two methods have been prescribed, the colorimetric method and the atomic absorption spectrometric method. The atomic absorption spectrometric method is the reference method.

F-1 COLORIMETRIC METHOD

F-1.1 Reagents

F-1.1.1 *Concentrated Ammonia*

F-1.1.2 *Sodium Citrate* — 10 percent.

F-1.1.3 *Dimethyl Glyoxime* — one percent in ethyl alcohol.

F-1.1.4 *Chloroform*

F-1.1.5 *Ammonia* (0.5 M) — 1 : 3.

F-1.1.6 *Ammonia* — 1 : 1.

F-1.1.7 *Hydrochloric Acid* — 0.5 M and 1 N.

F-1.1.8 *Sodium Hydroxide* — 2 N.

F-1.1.9 *Potassium Persulphate* — 2 percent.

F-1.1.10 *Standard Nickel Solution*

Dissolve 0.447 9 g of nickel sulphate ($NiSC_4$) ($6H_2O$) in water or 0.1 g of 99.9 percent pure nickel in 10 ml of nitric acid and when dissolution is complete, boil gently to expel oxides of nitrogen and dilute to 1 litre. This solution contains 0.1 mg/ml of nickel and may be further diluted to 0.01 mg/ml, if necessary.

F-1.1.11 *Perchloric Acid* — 6 percent.

F-1.1.12 *Bromine Water*

F-1.1.13 *Nitric Acid* — 1 N.

F-1.2 Procedure

F-1.2.1 *Preparation of Sample* — Representative sample to give a final solution containing greater than 0.05 but less than 10 µg Ni/ml is weighed into a silica dish and heated to flash point using a Bunsen Burner. When the oil catches fire, Bunsen Burner is removed and the oil is allowed to burn in a hood without exhaust. After about 30 minutes the flame ceases on its own and a very small quantity of residue remains. The residue is heated in low Bunsen flame till smoke ceases, before keeping the sample in the muffle furnace. Ashing is completed in the muffle furnace maintaining the temperature at 450°C. Ashing will be complete within 2 hours. Care is needed to take out the sample from the muffle furnace as the ash is very fluffy and quantity is small.

F-1.2.1.1 Dissolve the ash in 1 N nitric acid by warming on the water bath and filter through ashless Whatman filter paper and make up to suitable volume with 1 N nitric acid.

F-1.2.2 *Separation of Nickel by Dimethyl Glyoxime-Chloroform Extraction* — To an aliquot containing about 50 to 100 ppm of nickel add 5 ml of 10 percent sodium citrate. Neutralize with concentrated ammonia and add a few drops in excess (*p*H greater than 7.5). Add 2 ml of dimethyl glyoxime (or more as may be required if such copper or cobalt is present, that is, extra volume or 2 ml for each 10 mg of copper and 5 ml for each 10 mg of cobalt). Extract with three 2 to 3 ml portions of chloroform shaking for 30 seconds each time. Shake the combined chloroform extracts with 0.5 ml of 1:3 (0.5 M) ammonia. Repeat the wash with another portion of ammonia if much copper or cobalt is present. Shake the ammonia washings with 1 or 2 ml of chloroform and add the latter to the chloroform extract. Carry out reagent blank using the same quantity of reagents.

Return the nickel to the ionic state by shaking with two 5-ml portions of 0.5 M hydrochloric acid, transfer this solution to a 100-ml volumetric flask and proceed as per calibration.

F-1.2.3 *Colour Development*

F-1.2.3.1 *Method using persulphate for oxidation*

F-1.2.3.1.1 *Calibration* — Transfer 1, 2, 4, 6, 8 and 10 ml of standard nickel solution (1 ml - 0.01 mg Ni) to six 100-ml volumetric flasks. Add 5 ml of 1 N hydrochloric acid. Dilute to 50 ml and add 1 ml of 10 percent sodium citrate, 3 ml of 2 percent potassium persulphate solution, 15 ml of 2 N sodium hydroxide solution and finally 1 ml of 1 percent ethanolic dimethyl glyoxime solution. Heat to 60 to 70°C and keep at the temperature for 5 minutes. Cool to room temperature and dilute to volume. Measure the absorbance at 465 nm. Plot the photometric readings of the calibration solution against milligrams of nickel present in 100 ml solution.

F-1.2.3.1.2 Develop the colour for sample as described under calibration.

F-1.2.3.2 *Method using bromine for oxidation*

F-1.2.3.2.1 *Calibration* — Transfer 1, 2, 4, 6, 8 and 10 ml of standard nickel solution (1 ml-0.01 mg) to six 100-ml volumetric flasks. Add 2 ml of perchloric acid to each flask. Also prepare a separate reference solution by adding 2 ml of perchloric acid to a 100-ml volumetric flask. Dilute to 50 ml and add successively (swirling the flasks between additions) 10 ml of sodium citrate, 5 ml of bromine water and adjust sufficient ammonia (1:1) to bleach the bromine colour. Add 3 ml of ammonia (1:1) in excess and cool to room temperature. Add 3 ml of dimethyl glyoxime solution, dilute to the mark and mix (the addition of bromine water, ammonia and dimethyl glyoxime should be made without interruption and subsequent photometric measurement should be made after 10 ± 2 minutes after addition of dimethyl glyoxime). Transfer a suitable quantity of the solution to an absorption cell, cool and measure the absorbance at 530 nm. Plot the photometric readings of the calibration solution against milligrams of nickel per 100 g of solution.

F-1.2.3.2.2 Develop the colour for sample as described under calibration and read the absorbance against reagent blank.

F-1.2.4 *Calculation*

$$\begin{array}{c} \text{Concentration of nickel} \\ \text{in sample (ppm)} \end{array} = \begin{array}{l} \text{Measured} \\ \text{concentration} \\ \text{(from calibration} \\ \text{curve)} \end{array} \times \frac{\text{Dilution factor}}{\begin{array}{c} \text{Mass of sample} \\ \text{in g} \end{array}}$$

F-2 ATOMIC ABSORPTION SPECTROMETRIC METHOD

F-2.1 Reagents

F-2.1.1 *Stock Nickel Solution* — Dissolve 1 g of 99.99 percent nickel in minimum volume of 1 + 1 HNO_3 and dilute to one litre with 1 percent nitric acid.

F-2.1.2 *Nitric Acid* — 1N.

F-2.2 Procedure

F-2.2.1 *Preparation of Sample* — Representative sample to give a final solution containing greater than 0.05 but less than 10 µg Ni/ml is weighed into a silica dish and heated to flash point using a Bunsen Burner. When the oil catches fire, Bunsen Burner is removed and the oil is allowed to burn in a hood without exhaust. After about 30 minutes the flame ceases on its own and a very small quantity of residue remains. The residue is heated in low Bunsen flame till smoke ceases, before keeping the sample in the muffle furnace. Ashing is completed in the muffle furnace maintaining the termperature at 450°C. Ashing will be complete within 2 hours. Care is needed to take out the sample from the muffle furnace as the ash is very fluffy and quantity is small.

F-2.2.1.1 Dissolve the ash in 1 N nitric acid by warming on the water bath and filter through ashless Whatman filter paper and make up to suitable volume with 1 N nitric acid.

F-2.2.2 *Calibration of Atomic Absorption Spectrometer* — Calibrate tne instrument with 0.2, 0.4, 0.8, 1.6, 2.0, 4.0, 8.0 and 10 µg/ml standard nickel solution by setting the wavelength of 232 nm using air acetylene flame and 1N nitric acid as blank. The absorbance is plotted against concentrate.

F-2.2.3 *Measurement* — Run the sample solution into the instrument and calculate the concentration of nickel in the sample.

F-2.2.4 *Calculation*

$$\text{Concentration of nickel in sample (ppm)} = \text{Measured concentration} \times \frac{\text{Dilution factor}}{\text{Mass of sample in g}}$$

(FAD 44)

Indian Standard

SPECIFICATION FOR BAKERY SHORTENING

(First Revision)

0. FOREWORD

0.1 This Indian Standard (First Revision) was adopted by the Indian Standards Institution on 31 January 1986, after the draft finalized by the Oils and Oilseeds Sectional Committee had been approved by the Chemical Division Council and the Agricultural and Food Products Division Council.

0.2 The specifications for bakery shortening have been prescribed under the *Prevention of Food Adulteration* (*PFA*) *Rules*, 1955 and *Vegetable Oil Products* (*Standards of Quality*) *Order*, 1975 (VOP) issued by Ministries of Health and Civil Supplies, Government of India, respectively. A need was, however, felt to formulate an Indian Standard on this subject including its methods of sampling and test which are not included in the PFA Rules or *VOP* (*Standards of Quality*) *Control Order*, 1975.

0.3 This specification is primarily based on the requirements prescribed under PFA Rules and *Vegetable Oil Products* (*Standards of Quality*) *Order*, 1975 as amended from time to time. However, in line with the present practice, requirements for acid value and refractive index have been specified and their corresponding limits in terms of free fatty acids (FFA) and butyro refractometer (B.R.) reading have also been given.

0.4 This Indian Standard was first published in 1983 and is now being revised to incorporate number of modifications. Some of the changes made in this standard are as follows:

a) The requirement for using only refined grade of sesame oil conforming to IS : 547-1968* has been deleted;

b) In order not to unduly burden the testing laboratories the non-edible oils, the test for which should be carried in routing analysis, have been specified;

c) A range for the melting point requirement instead of an upper limit only has been stipulated;

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*Specification for sesame oil (*second revision*).

d) Requirement for only a positive test for Vitamin A in addition to the requirement for 25 I.U., when packed, has been prescribed;

e) The capillary slip method for determination of melting point and Carr Price method for determining positive test for Vitamin A have also been included in this revision;

f) The new definition for *vanaspati* to bring in line with PFA specification has been included; and

g) The requirement for antioxidants and synergists and the description clause have also been modified.

0.5 For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS : 2-1960*. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

1. SCOPE

1.1 This standard prescribes requirements and methods of sampling and test for bakery shortening.

2. TERMINOLOGY

2.1 For the purpose of this standard, the following definitions in addition to those given in IS : 11476-1985† shall apply.

2.1.1 *Bakery Shortening* — Bakery shortening means *vanaspati* meant for use as a shortening or leavening agent in the manufacture of bakery products for promoting the development of the desired cellular structure in the bakery product with an accompanying increase in its tenderness and volume.

2.1.2 *Vanaspati* — *Vanaspati* means any refined edible vegetable oil or oils subjected to a process of hydrogenation in any form.

NOTE — However, as per VOP, *vanaspati* means hydrogenated oil meant for human consumption.

3. REQUIREMENTS

3.1 Description — The material shall be clear in appearance on melting and free from sediments, suspended and other foreign matter, separated

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*Rules for rounding off numerical values (*revised*).

†Glossary of terms relating to oils and fats.

water, added colouring or flavouring substances or any other such substances or any matter deleterious to health. It shall have a characteristic odour and taste and shall be free from foreign odour and taste. Antioxidants and synergists, as permitted by the competent authority, may be added to improve its keeping property. It may also contain added monoglycerides and diglycerides or any other permitted emulsifying agents, and if aerated, only nitrogen, or air shall be used for the purpose and the quantity of such gas incorporated in the product shall not exceed 12 percent by volume.

3.1.1 The clarity of the material shall be judged by the absence of turbidity after keeping the filtered sample at 50°C for 24 hours.

3.2 Raw Materials — Only prescribed vegetable oils and in such specified proportions as modified from time to time by the enforcing authorities shall be used. These oils shall be free from non-edible oils when tested as per IS : 548 (Part 2)-1976*.

3.3 The material shall be prepared by a process of hydrogenation of only prescribed edible vegetable oils and in such specified proportion as modified from time to time by the enforcing authorities. Only those chemicals which can be totally removed and shall have no adverse effect on quality of the product, shall be used in the processing.

3.4 The material shall contain not less than 5.0 percent by mass of raw or refined sesame oil (conforming to IS : 547-1968†) but sufficient to ensure that when the material is mixed with refined groundnut oil in the proportion of 20 : 80, the red colour produced by the Baudouin test shall not be lighter than 2 red units in 1-cm cell on Lovibond scale when tested as prescribed in Appendix A.

3.5 The material shall be free from argemore oil, mineral oil, castor oil and animal fat when tested as prescribed in **10**, **12**, **15** and **18** of IS : 548 (Part 2)-1976*.

3.6 The material shall be manufactured in the premises maintained under hygienic conditions (*see* IS : 2491-1972‡).

3.7 The material shall also conform to the requirements prescribed in Table 1.

3.8 Optional Requirement — The solid fat index (dilation value) at 15°C shall be not more than 1 000 when tested according to the method given in Appendix E.

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*Methods of sampling and test for oils and fats: Part 2 Purity tests (*third revision*).

†Specification for sesame oil (*second revision*).

‡Code for hygienic conditions for food processing units (*first revision*).

TABLE 1 REQUIREMENTS FOR BAKERY SHORTENING

(*Clauses* 3.7 *and* 7.1)

Sl No.	Characteristic	Requirement	Method of Test, Ref to	
			Appendix	Cl No. in IS : 548 (Part 1)-1964*
(1)	(2)	(3)	(4)	(5)
i)	Moisture, percent by mass, *Max*	0·25	—	5
ii)	Acid value†, *Max*	0·5	—	7
iii)	Unsaponifiable matter, percent by mass, *Max*	2·0	—	8
iv)	Melting point, °C, *Max*	31-41	B	—
v)	Refractive index at 60°C‡, *Min*	1·450 5	—	10
vi)	Iodine value, *Max*	75	—	14
vii)	Synthetic vitamin A, expressed in international units (I.U.), per gram.			
	a) When packed, *Min*	25	C	—
	b) At retail outlet	Shall pass the test	D	—

*Methods of sampling and test for oils and fats: Part 1 Sampling, physical and chemical tests (*revised*).

†The corresponding figure in terms of free fatty acids (FFA) when expressed as oleic acid shall be maximum 0·25 percent by mass.

‡This corresponds to butyro refractometer (B.R.) reading of 37·4.

4. PACKING

4.1 The material shall be packed in suitably sealed packages, as agreed to between the purchaser and the supplier and as notified from time to time by the enforcing authorities.

5. MARKING

5.1 The container shall be marked with the following particulars:

a) Name of the material;

b) Manufacturer's name and his recognized trade-mark, if any;

c) Net mass in the container;

d) The words ' contains 25 I.U. of vitamin A per gram when packed ';

e) Name and quantity of emulsifier;

f) Month and year of manufacture;

g) Batch number or lot number in code or otherwise; and

h) Any other marking specified by the statutory authority.

5.1.1 The product may also be marked with Standard Mark.

5.1.2 The use of the Standard Mark is governed by the provisions of the Bureau of Indian Standards Act, 1986 and the Rules and Regulations made thereunder. The details of conditions under which the licence for the use of Standard Mark may be granted to manufactures or producers may be obtained from the Bureau of Indian Standards.

5.2 In addition, the following information shall be suitably marked, either printed on the label affixed to the container, or lithographed or stencilled thereon with indelible ink, for example, in a type size of not less than 12·5 mm in case of 15 kg tin containers:

'MADE FROM VEGETABLE OILS ONLY'

6. SAMPLING

6.1 Representative samples of the material shall be drawn as prescribed under **3** of IS : 548 (Part 1)-1964*.

6.2 Criterion for Conformity — A lot shall be declared conforming to the requirements of this standard if the test results on the composite samples satisfy the requirements prescribed under **3**.

7. METHODS OF TESTS

7.1 Tests shall be carried out according to the methods prescribed in IS : 548 (Part 1)-1964*, and IS : 548 (Part 2)-1976† for the characteristics given in Table 1 (*see* **3.7**) and Appendices A, B, C, D and E.

7.2 Quality of Reagents — Unless specified otherwise, pure chemicals and distilled water (*see* IS : 1070-1977‡) shall be used in the test.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the result of analysis.

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*Methods of sampling and test for oils and fats: Part 1 Sampling, physical and chemical tests (*revised*).

†Methods of sampling and test for oils and fats: Part 2 Purity tests (*third revision*).

‡Specification for water for general laboratory use (*second revision*).

APPENDIX A

(*Clauses* 3.4 *and* 7.1)

TEST FOR THE PRESENCE OF SESAME OIL (BAUDOUIN TEST)

A-0. GENERAL

A-0.1 Outline of the Method — The development of a permanent pink colour with furfural solution in presence of hydrochloric acid indicates the presence of sesame oil.

A-1. REAGENTS

A-1.1 Refined Groundnut Oil — (*see* IS : 544-1968*) showing negative Baudouin test.

A-1.2 Dilute Hydrochloric Acid — relative density 1·125.

A-1.3 Concentrated Hydrochloric Acid — fuming, relative density 1·19.

A-1.4 Furfural Solution — 2 percent solution of furfural, distilled not earlier than 24 hours prior to the test in rectified spirit (conforming to IS : 323-1959†). The reagent is stable up to 3 months kept in a refrigerator.

A-2. APPARATUS

A-2.1 Lovibond Tintometer

A-2.2 Measuring Cylinder — 25-ml capacity.

A-3. PROCEDURE

A-3.1 Melt the sample of the vegetable oil product, that is, the bakery shortening, completely and mix it well at a temperature of about 50°C.

A-3.2 Check for the presence of colouring matter which are chromogenic in the presence of hydrochloric acid, by the method given in **A-3.2.1** and **A-3.2.2**.

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*Specification for groundnut oil (*second revision*).

†Specification for rectified spirit (*revised*).

A-3.2.1 Shake 10 ml of the melted bakery shortening with 10 ml concentrated hydrochloric acid. Note if any red colour develops in the aqueous layer.

A-3.2.2 If a red colour develops in the aqueous layer, shake 20 ml of the melted bakery shortening in a separating funnel for half-a-minute with 15 ml of dilute hydrochloric acid. During the treatment, do not permit the temperature of the contents of the separating funnel to exceed that temperature necessary to keep the sample in liquid condition. Draw off the red acid layer which collects at the bottom of the funnel and repeat the process until no further colouration takes place.

A-3.3 Dilute 20 ml of the melted bakery shortening, either the original sample, if no red colour develops on checking as in **A-3.2.1**, or after complete removal of the hydrochloric acid layer as in **A-3.2.2**, with 80 ml of refined groundnut oil. Take 5 ml of this mixture in a 25-ml measuring cylinder with glass stopper and add 5 ml of concentrated hydrochloric acid. Add 0·4 ml of the furfural solution, shake vigorously for 2 minutes and allow to stand for 5 minutes. Transfer the contents of the measuring cylinder to a separating funnel and decant the acid layer through a wet filter paper into a 1-cm Lovibond cell, cleaned with carbon tetrachloride and dried. Place the cell in position in a Lovibond Tintometer and viewing through the eyepiece of the instrument, match the colour shade of the filtrate with the appropriate combination of red and yellow slides. The colour, which is recorded in terms of red units only (correct to one place of decimal) shall be read within 5 minutes of the addition of furfural solution.

A-3.4 Perform a blank experiment using 5 ml of refined groundnut oil in place of the mixture and determine the colour. The Baudouin test reading of the sample shall be the colour reading given by the sample in **A-3.3** *less* that given by the blank.

APPENDIX B

[*Table* 1, *Item* (iv) and *Clause* 7.1]

DETERMINATION OF MELTING POINT OF BAKERY SHORTENING

B-1. APPARATUS

B-1.1 Melting Point Tubes — Thin-walled, uniformly-bored capillary glass tubes, open at both ends and having the following dimensions:

a) length, 50 to 60 mm;

b) inside diameter, 0·8 to 1·1 mm; and

c) outside diameter, 1·2 to 1·5 mm.

B-1.2 Thermometer — Centigrade, with 0·2° sub-divisions and a suitable range. The thermometer should be checked against a standard thermometer which has been calibrated and certified by the National Physical Laboratory, New Delhi.

B-1.3 Beaker — with a side-tube heating arrangement.

B-1.4 Water Bath — maintained at 15-17°C.

B-1.5 Heat Source — Gas burner or spirit lamp.

B-2. PROCEDURE

B-2.1 Melt the sample of the bakery shortening completely and mix it well at a temperature of about 50°C. Insert the melting point tube (thoroughly cleaned and dried before use) into the molten product so that a column of the product about 10 mm long, is forced into it. Allow the sample in the tube to just set, by keeping the tube in a horizontal position during winter; during summer, the tube may be put on a perforated metal tray which is so placed inside the water bath (at 15-17°C) that the bottom of the tray just touches the water. Then place the tube in a test-tube immersed in water at 15-17°C for one hour. Remove the melting point tube and attach it with a rubber band or any other suitable means to the thermometer so that the lower end of the melting point tube is even with the bottom of the bulb of the thermometer. Pour water at about 20°C into the beaker (with side-tube heating arrangement) and suspend the thermometer in the centre of the beaker so that the lower end of the sample column is 30 mm below the surface of water. Heat the side-tube of the apparatus gently, so that the temperature of water increases slowly at the rate of 2°C per minute till the temperature reaches 30°C and thereafter at the rate of 0·5°C per minute. Note the temperature of the water when the sample column commences to rise in the tube which is the melting point of the sample.

APPENDIX C

[*Table* 1, *Item* (vii) and *Clause* 7.1]

DETERMINATION OF VITAMIN A

C-1. GENERAL

C-1.1 Principle — The content of vitamin A is determined by measurement of the ultraviolet absorption spectrum of a fraction, in which the vitamin A alcohol is collected after its isolation by chromatography. It is expressed in International Units per gram.

C-1.1.1 One International Unit (I.U.) of vitamin A is equivalent to 0·3 µg vitamin A alcohol or 0·344 µg vitamin A acetate.

NOTE — The official method in use in the United Kingdom (UK), involves a somewhat different procedure to that given in this method but both techniques give the same result for vitamin A.

C-2. REAGENTS

C-2.1 Potassium Hydroxide — 50 percent aqueous solution.

C-2.2 Ethanol — 96 percent (*v/v*).

C-2.3 Diethyl Ether — peroxide-free.

NOTE — Make diethyl ether peroxide-free by distillation over potassium hydroxide. Store the peroxide-free ether over coarse granular carbon.

C-2.4 Petroleum Ether — distilled over potassium hydroxide, boiling range 40 to 60°C.

C-2.5 Alumina (Activated) — Heat the alumina at 600°C for 6 hours, cool, sieve through a 180 mesh sieve and add about 3 percent water. Mix thoroughly and allow the product to stand for at least 12 hours before use. Store in an air-tight bottle.

C-2.6 Alumina (Alkaline) — The same alumina as given in **C-2.5** is treated with sodium hydroxide as follows:

Stir 10 g alumina with a solution of 1 g sodium hydroxide in 10 ml water. Allow to stand at room temperature for one hour in a closed bottle and shake occasionally. Then heat in a dish in a vacuum drying oven at 100°C and 20 mmHg for 2½ hours. Pour the dried product into a bottle without removing any powder clinging to the wall of the dish and stopper securely. To the cooled powder, add 2 percent water and mix thoroughly. Allow to stand for 18 hours and determine the moisture content after drying for one hour in a drying oven at 105°C; if lower than 2 percent, again add water, mix and repeat the process.

C-2.7 Antimony Trichloride Solution — With a porcelain spoon, introduce 65 g of antimony trichloride into a 500-ml conical flask and wash several times with 15 ml chloroform until the chloroform remains clear. Then dissolve the antimony trichloride in 200 ml of chloroform by refluxing. Transfer the warm solution to a bottle containing anyhydrous sodium sulphate.

NOTE — The chloroform to be used should be washed with water and dried before use to remove any alcohol which may have been added to improve its keeping properties.

After some days antimony trichloride crystals are formed at the bottom and the wall; the solution is then quite clear and ready for use.

C-2.8 Acetone — conforming to IS : 170-1976*.

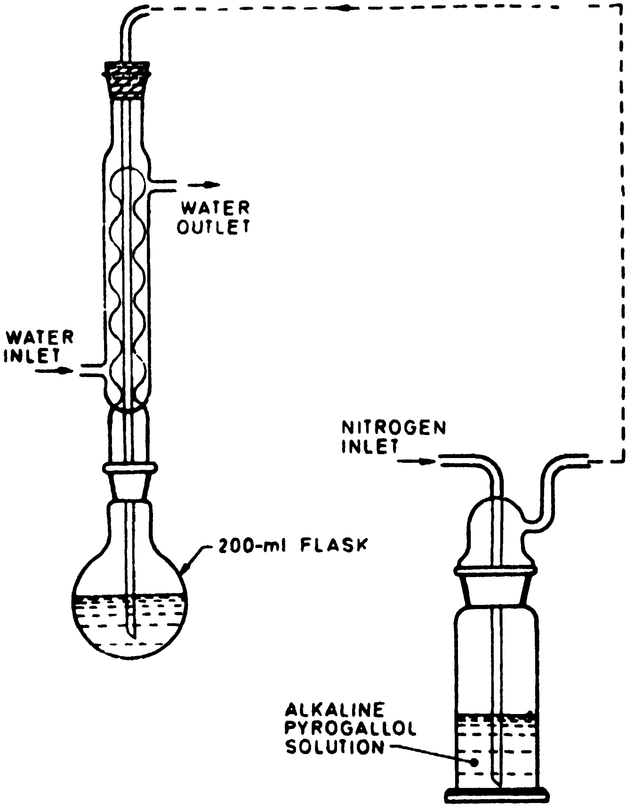

FIG. 1 APPARATUS FOR SAPONIFICATION

C-3. APPARATUS

C-3.1 Conical Flask — 50 ml.

C-3.2 Separating Funnels — 500 ml.

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*Specification for acetone (*second revision*).

C-3.3 Round Bottom Flask — 200 ml with ground glass stopper and two condensers.

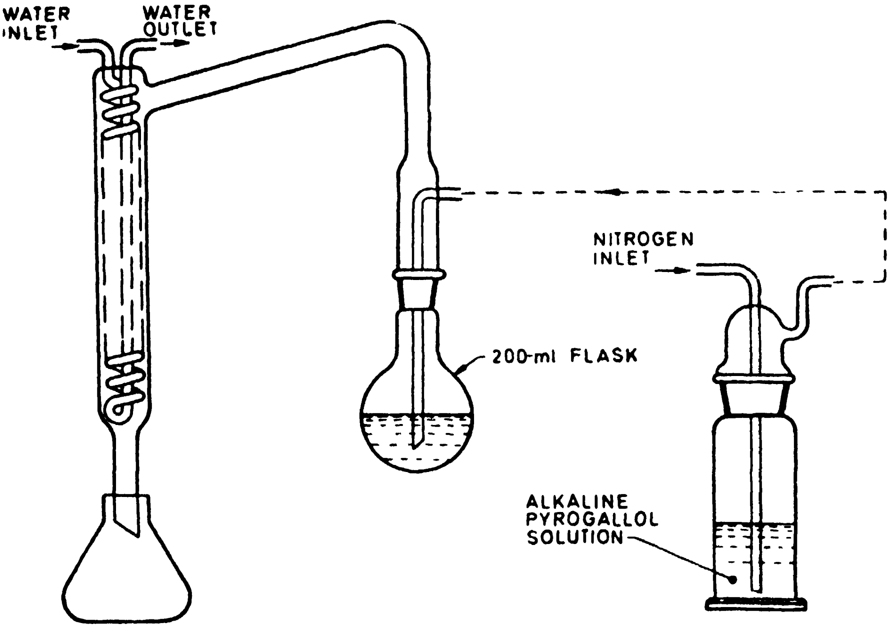


FIG. 2 APPARATUS FOR EVAPORATION

C-3.4 Chromatographic Apparatus (Fig. 3 to 6) — The apparatus consists of two parts, each containing a chromatographic column, which may readily be connected in series by means of a rubber stopper connection. The upper tube contains alumina, the lower alkaline alumina. Close both the tubes at the bottom with a plug of cotton wool. Fill the longer tubes with petroleum ether to a level which reaches into the widened section, and then very regularly and gently pour out the alumina, to a column height of 15 cm. Fill the shorter column, shortly before use, in the same way to a height of 2 cm with alkaline alumina.

C-3.5 Tubes — calibrated at 1-ml (*see* Fig. 5).

C-3.6 Pipette — 1-ml with a fine tip.

C-3.7 Graduated Flask — 10-ml.

C-3.8 Ultraviolet Spectrophotometer

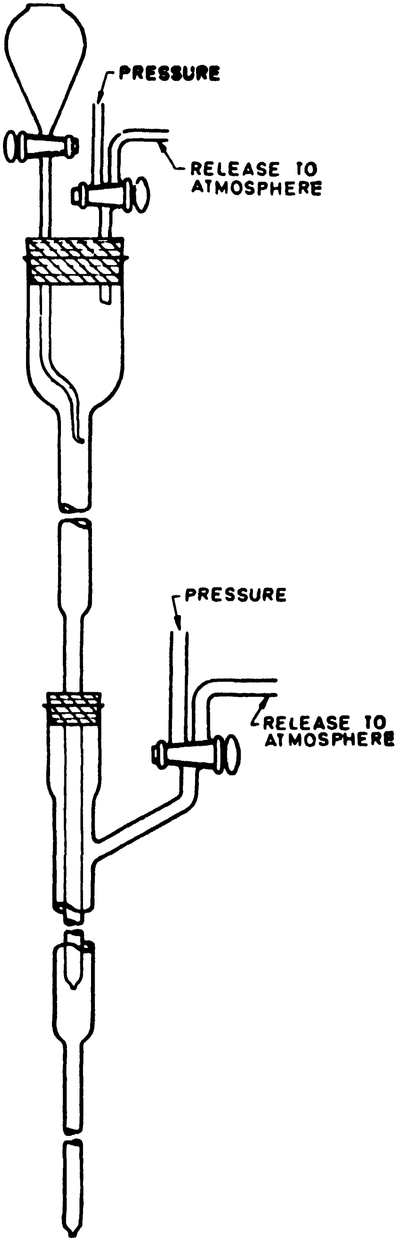


Fig. 3 Upper Tube

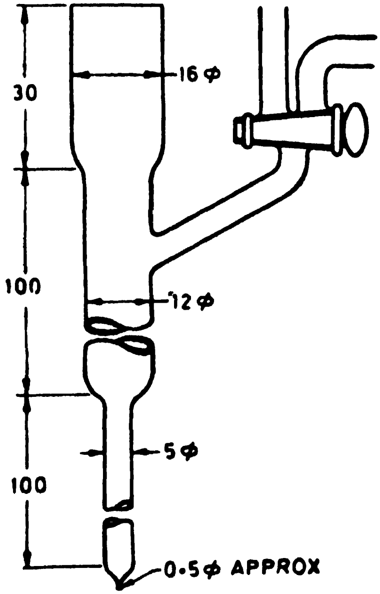


Fig. 4 Lower Tube

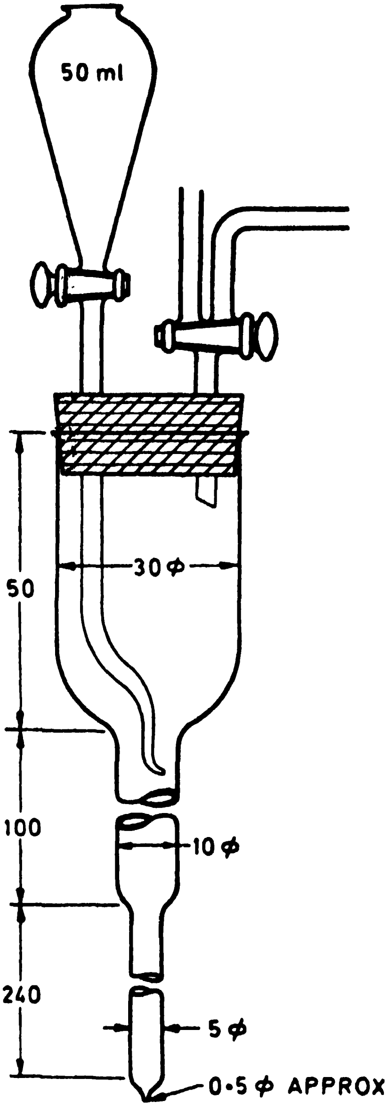


FIG. 5 RECEIVING TUBE

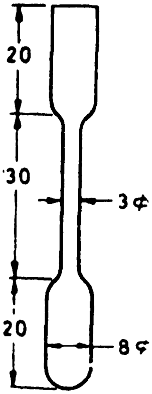


FIG. 6 ASSEMBLY OF CHROMATOGRAPHIC TUBE

C-4. PROCEDURE

C-4.1 Sampling — Take a sample of the material weighing approximately 10 g. Such a sample contains a total of about 250 I.U. vitamin A.

C-4.2 Saponification — Put the sample into the 200-ml round bottom flask and weigh to the nearest 50 mg. Add 8 ml of potassium hydroxide solution and 25 ml ethanol. Heat gently on a steam bath for one hour or until the saponification is complete, with a reflux condenser attached to the flask. During this procedure, pass a slow current of oxygen-free nitrogen through the liquid.

C-4.3 Extraction of Vitamin A — Add 50 ml water through the condenser and cool the soap solution in tap water. Transfer the solution to a separating funnel, using another 50 ml of water to rinse the flask. Extract the soap solution with successive portions of 100 and 50 ml diethyl ether by shaking. Wash the combined extracts in another separating funnel four times with 50 ml of water (the first time only by swirling, the following three times by gentle shaking). Continue washing if the ether layer is still turbid.

C-4.4 Evaporation of the Solvent — Use the same flask in which the saponification was carried out. Add the diethyl ether solution in two portions. Heat on the water-bath (80 to 85°C). During the distillation, maintain a weak current of oxygen-free nitrogen until approximately 5 ml is left. Then transfer the residue to a 50 ml conical flask, and rinse with 15 to 20 ml ether. Evaporate the ether with nitrogen, add some acetone and again evaporate the solvent.

C-4.4.1 Immediately take up the residue in 1-2 ml petroleum ether (if not clear, dry again with acetone) and introduce the solution on top of the upper chromatographic column with as little petroleum ether as possible (about 5 ml).

C-4.5 Chromatographic Separation of Vitamin A Alcohol — The chromatographic separation is carried out in two stages. For the first stage, only the upper column is used (alumina). After the petroleum ether extract (from **C-4.4.1**) has been brought on to the column, this is passed through the adsorbent. Rinse the conical flask with 5 ml petroleum ether and bring this amount on to the column. Rinse the flask with 5 ml portions of petroleum ether containing 4, 8 and 12 percent diethyl ether (*v/v*) respectively and use the washings for elution process. Discard all the elutes containing substances which are less strongly absorbed than vitamin A alcohol.

NOTE — Test for vitamin A with antimony trichloride in the elute containing 12 percent diethyl ether. If the reaction is positive (in which case the alumina contains too much water), repeat the determination with a fresh column packing.

C-4.5.1 Then connect the second column (alkaline alumina) and elute with 5 ml each of petroleum ether containing 16, 20 and 24 percent diethyl ether (v/v), respectively. Finally, use petroleum ether containing 36 percent diethyl ether (v/v) until the vitamin A has been eluted completely. Collect the eluted fractions in the tubes graduated at 1 ml. First, thoroughly mix the contents of each tube in order to obtain a homogeneous solution. Do this by blowing some air bubbles through by means of the fine-tipped pipette. Then with the same pipette, remove from each of the tubes approximately 0·3 ml of the solution and with these samples carry out the Carr-Price spot test (using small test tube) with 0·5 ml antimony trichloride solution and 1 drop of acetic anhydride.

NOTE — Ensure that during elution the columns do not run dry but at the same time prevent, as much as possible, the various petroleum ether fractions from mixing at the top of the first column. Only very little elute originating from the first column may be present on top of the second column.

C-4.5.1.1 Discard the fractions in which the Carr-Price spot test is negative [generally those with 16 and 20 percent diethyl ether (v/v)].

NOTE — Special care should be taken in carrying out Carr-Price spot test as the reaction is instantaneous (takes 3 to 6 seconds only) and the blue colour developed is very unstable. It is, therefore, recommended that addition of antimony trichloride reagent should be done quickly using blunt tipped pipette.

C-4.6 Spectrophotometric Determination of Vitamin A — Pipette exactly 0·5 ml from each tube in which the Carr-Price spot test is positive. Pool these in the 10-ml graduated flask, make up to the mark with petroleum ether used and mix. Make sure that the absorption at 326 μm of the petroleum ether used does not change when 10 percent diethyl ether is added. Use petroleum ether in the blank cell. Read the optical density at the top of the extinction curve (324 to 326 μm) using a 1-cm cell.

NOTE — It is recommended to occasionally compare the shape of the optical density curve between 260 and 370 μm with Morton-Stubbs' ideal curve. This is done to establish whether the separating capacity of the columns is sufficient. The freshly prepared adsorbents should always be tested in this way.

C-5. CALCULATION

$$\text{Vitamin A (I.U./g)} = \frac{366a}{M}$$

where

M = mass in g of sample, and

a = optical density reading at the maximum.

APPENDIX D

[*Table* 1, *Item* (vii) (b), and *Clause* 7.1]

QUALITATIVE DETERMINATION OF VITAMIN A (ANTIMONY TRICHLORIDE METHOD)

D-0. PRINCIPLE

D-0.1 The sample is saponified with ethanolic potassium hydroxide and extracted with petroleum ether. The extract is reacted with antimony trichloride and observed for appearance of a blue colouration which indicates the presence of vitamin A.

D-1. REAGENTS

D-1.1 Absolute Ethanol — Ethanol may be purified as follows:

Reflux absolute ethanol for 30 minutes in presence of 10 g caustic potash and 10 g aluminium powder per litre of ethanol. The mixture is then distilled in an all-glass apparatus with a fractionating column of 50 to 60 cm length. Collect only middle fraction of the distillate, discarding the first and the last fraction each amounting to 5 percent of the total volume. The ethanol thus obtained should have the same optical absorption as glass distilled water in ultraviolet region.

D-1.2 Potassium Hydroxide Solution — 50 percent (*m*/*v*).

D-1.3 Ether — peroxide free, redistilled (*see* IS : 336-1964*). Ether may be maintained free from peroxides by adding wet zinc foil approximately 80 cm^2 per litre, cut in strips long enough to reach at least half way up the container, the zinc strips previously should have been completely immersed in dilute acidified copper sulphate solution for one minute and subsequently washed with water.

D-1.4 Sodium Sulphate — anhydrous granular. It shall not absorb vitamin A under conditions of use, and 10 percent solution shall not be acidic to methyl red indicator solution.

D-1.5 Chloroform — This may be purified as follows:

Wash the chloroform thrice with fresh 10 percent aqueous solution of sodium thiosulphate in a separatory funnel. Dry the chloroform with anhydrous calcium chloride and filter. Distil the chloroform over anhydrous sodium thiosulphate in an all-glass apparatus with a fractionating column of 70 cm length. Collect only middle fraction of the distillate, discarding the first 10 percent and last 10 percent of the distillate.

---

*Specification for ether (*revised*).

Anhydrous sodium thiosulphate may be prepared from crystalline thiosulphate by careful heating between 105°C to 110°C and storing in a desiccator.

D-1.6 Antimony Trichloride Solution — Prepare by dissolving 113·4 g antimony trichloride in 300 to 400 ml of chloroform. Add 5 g of anhydrous calcium chloride and filter while hot. Dilute the filtrate to 500 ml with chloroform.

D-2. PROCEDURE

D-2.1 Weigh approximately 5 g of the material and transfer to an all-glass saponification flask, if necessary with a little alcohol. Add 30 ml of ethanol (95 percent *v/v*) and 5 ml of potassium hydroxide solution with a jet tube for 5 minutes to replace the air. Then in a dark corner, saponify the contents under reflux in an atmosphere of nitrogen over an electrically heated water bath for 30 to 40 minutes. Cool and then add 30 ml of water. Transfer the contents quantitatively to a separating funnel (conical, squibb shaped, 250-ml capacity). Wash the flask twice, with 10 ml of water and add the washings to the separating funnel. Extract the saponified solution thrice, respectively with 50, 20 and 20 ml ether; combine the ether extract in another separating funnel and wash this with 50 ml of ice cold water containing a little sodium acid sulphate ($NaHSO_4$) or potassium acid phosphate (KH_2PO_4) just enough to neutralize the alkalinity of the extract. Repeat the washing with only ice cold water till the washings are neutral to phenolphthalein.

Transfer the ethereal extract to a stoppered measuring cylinder (100 ml), rinse the separating funnel with a little ether and then add this to the cylinder. Add more ether to make the volume to 100 ml mark. Then add anhydrous sodium sulphate (15 grams), mildly shake a little and allow it to settle in a dark cool place. Evaporate the ether under moderate heat and reduced pressure to obtain a residue. Dissolve the residue in a small amount of chloroform. Take 2 ml of the chloroform solution, add a drop of acetic anhydride and then add rapidly 0·5 ml of antimony trichloride solution.

D-2.2 The material shall be considered to have passed the test if a blue colouration appears immediately indicating the presence of vitamin A.

NOTE — Special care should be taken in carrying out this test since the reaction is spontaneous (takes 3 to 6 seconds only) and the blue colour developed is very unstable.

APPENDIX E

(*Clause* 7.1)

TEST FOR SOLID FAT INDEX

E-0. GENERAL

E-0.1 Solid fat index (dilation value) is an empirical measure of the solid fat content. It is calculated from the specific volumes at various temperatures utilizing a dilatometric scale graduated in units of ml × 1 000. Results are, therefore, expressed as melting dilation in ml/kg of fat.

E-0.2 The method is applicable to margarine oils, shortenings and other fats with a solid index of 50 or less at 10°C. The method is empirical and departures from the procedures may cause variations in results.

E-1. APPARATUS

E-1.1 Resistant Borosilicate Glass Dilatometers — Constructed in accordance with the specifications given in Fig. 7. The stem should be made from precision bore capillary tubing graduated in 0·005-ml increments from 0 to 1·400 ml, with an overall accuracy of at least ± 0·005 ml. The scale shall be marked 0 to 1·400 in intervals of 50. The dilatometers shall have identification numbers on the stems and stoppers.

E-1.2 Springs — To fasten dilatometer stoppers.

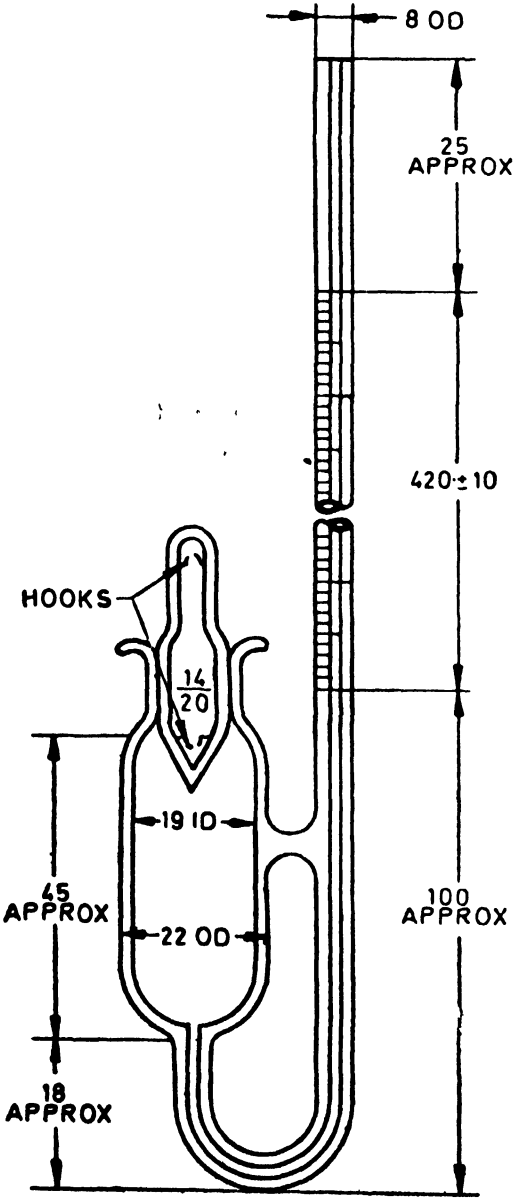
E-1.3 Clamps — For holding the dilatometers in the constant temperature baths.

E-1.4 Constant Temperature Water Baths — accurate to ± 0·05°C equipped to provide adequate circulation.

E-1.5 Vacuum Pump — capable of reducing pressure to 2 mmHg or less.

E-1.6 Stopcock — 2 mm ID capillary 2-way stopcocks with a burette tip made of borosilicate glass.

E-1.7 Cement



All dimensions in millimetres.

FIG. 7 DILATOMETER

E-2. REAGENTS

E-2.1 Potassium Dichromate Indicator Solution – one percent in water.

E-2.2 High Vacuum Grease -- silicone type.

E-2.3 Petroleum Solvent

E-2.4 Mercury -- distilled.

E-3. CALIBRATION

E-3.1 All new dilatometers shall be checked for accuracy as follows:

a) Thoroughly clean and dry the dilatometer;

b) Clamp the dilatometer securely in an inverted position;

c) Clamp the capillary stopcock in place at the end of the dilatometer stem and make a seal with cement;

d) After the cement has hardened, immerse the tip of the stopcock into a reservoir of clean mercury which is at room temperature;

e) Using vacuum, draw the mercury into the dilatometer stem until the calibrated portion is full;

f) Successively withdraw 0·200 ml portions of mercury into a tared 50-ml beaker and record the mass; and

g) Calculate the true volume in ml contained in each measured scale intervals as follows:

$$\frac{\text{mass of mercury}}{\text{(final — initial) scale reading}} \times \text{sp vol of mercury at } T_R \times 1\,000$$

where

T_R = room temperature.

1 ml = 1 000 in scale reading.

NOTE — In order to meet the specifications of this method, the dilatometer scale should be accurate to 0·005 ml or less (1 scale graduation) from 0 to 1 400. It is necessary to draw correction curves from the calibration data for those dilatometers which do not meet specifications and corrected reading must be used to calculate the solid fat index.

E-4. PROCEDURE

E-4.1 Filling the Dilatometer

E-4.1.1 Deaerate about 50 ml of the indicator solution for 3 minutes in a 250 ml filter flask or a strong oil sample bottle at a pressure slightly above the vapour pressure of the solution at the temperature of deaeration. (The vapour pressure of water at 25°C is 24 mm). The indicator may also be deaerated by vigorously boiling for 15 minutes at atmospheric pressure but should be cooled to room temperature before use.

E-4.1.2 Heat the bakery shortening sample to 80°C and deaerate in a 250 ml filter flask or strong sample bottle at a pressure of 2 mmHg until no more gas bubbles are seen, for at least 2 minutes. The sample must be maintained in a liquid state and agitated vigorously during deaeration.

NOTE — The indicator and sample should be used as soon as possible after they have been deaerated. The fat should be completely melted. Even slight crystallization occludes air.

E-4.1.3 Pipette 2 ml of the indicator solution into the dilatometer bulb. Lubricate the stopper lightly with silicone grease and weigh the assembled dilatometer to the nearest 0·01 g on a torsion balance.

E-4.1.4 Carefully overlay the indicator with the melted sample and fill until the sample overflows. Insert the stopper so that the indicator solution rises to approximately the 1 200-mark of the stem when the stopper is securely sealed. (The reading should be 1 200 ± 100 at 60°C; if not, the determination should be repeated).

E-4.1.5 Wash the fat from the outer surface of the dilatometer with petroleum solvent. Attach the retaining springs and reweigh the dilatometer when the solvent has evaporated.

E-4.2 Measurement of Thermal Expansion

E-4.2.1 Immerse the dilatometer to the 300-mark in the 60°C bath and record reading after 15 minutes and then at 5 minute intervals until the change is less than 2 units in 5 minutes. (Rechecks of the 60°C reading at the end of the determination should agree with the 60°C reference reading). Significant variations indicate faulty technique.

E-4.2.2 Transfer the dilatometer to the 37·8°C bath and immerse to the 300-mark. Read level of indicator at intervals of 5 minutes until the change is less than 2 units in 5 minutes. Record the readings.

NOTE — It is necessary for the sample to be completely melted at the lower temperature. If any seeding or clouding of the sample occurs, remelt the sample in a 60°C bath and raise the temperature of the other bath. If the reference bath temperatures are changed, appropriate substitution must be made in the calculations.

E-4.3 Conditioning of the Sample

E-4.3.1 Transfer the dilatometer to the 0°C bath and immerse to the 300-mark and hold for 15 minutes.

E-4.3.2 Transfer to a 15·0°C bath and hold for 30 minutes.

E-4.3.3 Transfer back to 0°C bath and hold for 15 minutes.

NOTE — If an ice bath is used, provisions should be made for adequate water circulation.

E-4.4 Measurement of Dilation

E-4.4.1 Transfer the dilatometer from the 0°C bath to a bath at the lowest desired temperature. Immerse to the 300-mark and record reading at 30 minutes.

E-4.4.2 Repeat at the next highest temperature and so on until readings have been obtained at all of the desired temperatures.

E-4.5 Calculation

$$\text{Solid fat index (at } 15^\circ\text{C)} = (\text{Total dilation}) - [\text{Thermal expansion} \times (60 - 15)]$$

$$\text{Thermal expansion of sample per degree C in ml/kg} = \frac{R_{60} - R_{15} - VC_{15}}{M \times (60 - 15)}$$

$$\text{Total dilation between } 15^\circ\text{C and } 60^\circ\text{C in ml/kg} = \frac{R_{60} - R_{15} - VC_{15}}{M}$$

where

VC_{15} = volume correction for expansion of glass and water at 15°C;

R_{15} = dilatometer reading at 15°C;

R_{60} = dilatometer reading at 60°C; and

M = mass in g of the sample.

NOTE 1 — *VC* from the Table 2 represents the combined corrections for the expansion of glass and water and applies to borosilicate glass only. If dilatometer is made of glass other than borosilicate, the corrections should be redetermined.

NOTE 2 — The liquid thermal expansion is basic for calculating the solid fat index. It should, therefore, be accurate.

Repeat analyses have shown values of 0·83 to 0·85 ml/kg to be normal for cottonseed oil, soyabean oil, lard and tallow. Lauric acid oils, such as coconut, have values of 0·85 to 0·87 ml/kg. If determined values are abnormal, the analyses should be repeated. Standard thermal expansions may be applied in routine determinations where results are used within an organization, provided they are checked periodically by actual measurement.

TABLE 2 VOLUME CORRECTIONS

BATH TEMPERATURE °C	60°C READING				
	1 000	1 100	1 200	1 300	1 400
0	22·0	20·3	18·6	16·9	15·2
5	22·2	20·5	18·7	17·0	15·3
10	21·8	20·1	18·4	16·7	15·1
15	21·0	19·5	17·8	16·2	14·6
20	19·8	18·4	16·8	15·3	13·8
25	18·4	17·0	15·6	14·1	12·7
30	16·6	15·3	14·0	12·7	11·4
35	14·4	13·3	12·2	11·1	10·0
40	12·0	11·0	10·2	9·2	8·3
45	9·4	8·7	8·0	7·2	6·5
50	6·6	6.1	5·6	5·1	4·5
55	3·2	3·0	2·8	2·5	2·3
60	0	0	0	0	0

(Continued from page 2)

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